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13. ABSTRACT ***(Maximum 200 Words)***

Two major independent barriers against the successful therapy of breast cancer are mutation of the tumor suppressor p53 gene and overexpression of the c-erbB-2/neu gene. However, there is little or no information on how, if at all, these molecular defects together influence therapeutic outcome. Of further concern is the absence of any therapeutic agents that could be used against both defects. The present research project was proposed to address these limitations.

The results from this project indicate that both p53 (wild-type and mutant) and overexpression of c-erbB-2/neu lead to cisplatin resistance, and that the resistance due to wild-type p53 and c-erbB-2/neu overexpression can be circumvented by DACH-acetato-Pt. The fact that under certain cellular context, wild-type p53 can lead to substantially greater resistance to an antitumor agent is a novel finding that may have greater limitations in the treatment of breast cancer. The data further indicate that overexpression of c-erbB-2/neu can interfere with p53 regulation when the DNA damaging agent is cisplatin, but there is no effect on regulation when the damage is induced by DACH-acetato-Pt. This suggests that the novel compound may have clinical utility in the treatment of breast cancer.

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Introduction

Two of the major barriers against the successful therapy of breast cancer are mutation of the tumor suppressor p53 gene and overexpression of the c-erbB-2/neu gene. These conclusions have been drawn from many studies reported, individually for each of the genetic defects, over the last decade. However, there is little or no information on how, if at all, these genes influence one and other in the treatment outcome with therapeutic antitumor agents. The end result is that treatment decisions are made in absence of any objective information to guide the way. Of further concern is the absence of any therapeutic agents that could be used under such circumstances. The present research project was proposed to address these limitations. During our first year of support from the US Army, we have generated evidence that wild-type p53 may over-ride the negative effects of c-erbB-2/neu overexpression, but only in conjunction with a specific platinum agent DACH-acetato-Pt.

Body of Report

During the first year of this project, aspects of Tasks 1, 2, 3, 6, and 7 were addressed concurrently for greater efficiency.

Task 1

We have identified several breast tumor models for use in the project, and have made progress in characterizing their status with regard to p53 and c-erbB-2/neu. This Task is still ongoing as attempts to identify and confirm a model with a characteristic of having null p53 and overexpressing c-erbB-2/neu was unsuccessful. However, we have selected the MDA-MB-157 cell line as a substitute. Since this line has p53-null status and lacks c-erbB-2/neu overexpression, we aim to achieve our objectives by first transfecting MDA-MB-157 cells with either a wild-type or a temperature-sensitive p53 vector. The option of a temperature-sensitive option is necessary since it is possible that wild-type p53 transfectants may be non-viable. Once stable clones expressing p53 have been selected, we will introduce the c-erbB-2/neu gene into the transfectant cell line to generate double-insert clones. As a back-up option, we have already tarnsfected the p53-nnull and c-erbB-2/neu overexpressing SKOV-3 cell line with a temperature-sensitive p53 vector and are in the process of characterizing the clones. Although the SKOV-3 is a cell line of ovarian origin, the etiology of this disease is similar to breast cancer and may provide significant information.

The other models that have been selected for the studies are listed in Table 1. The translational product of c-erbB-2/neu expression is a protein p185, the abundance and phosphorylation status of which has been assessed by Western blot for selected models. In Figure 1, the low expression of p185 and its active phosphorylated form is apparent for the MCF-7 parental cells and its "neo" control clone. In contrast, the MCF-7/Her2-18 and SK-Br3 express substantial high levels of p185 and its active form. There is an indication of an increase in Mdm2 levels in the MCF-7/Her2-18 model compared to the parental or neo control cells, and this may influence wild-type p53 function. The B-actin levels demonstrate equivalency of protein loading on the gel. These data validate the expected c-erbB-2/neu status of the models.

Table 1. Characteristics of breast tumor models

Model	MCF-7 par	MCF-7/neo	MCF-7/Her2-18	ZR75-1	MDA-MB-435 par	MDA-MB-435/neo	MDA-MB-435/eB1	SK-Br3
p53 status	wild-type	wild-type	wild-type	wild-type	mutant	mutant	mutant	mutant
c-erbB-2/neu overexp	low	low	high	low	no	no	high	high

par = parental cell line.

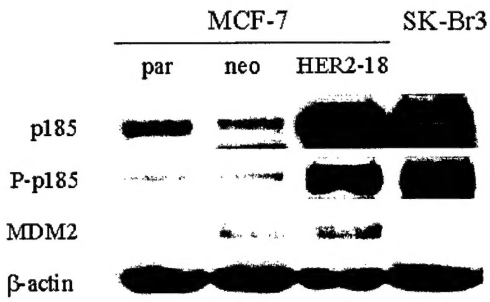

Figure 1. Western immunoblot of basal levels of p185 and associated proteins.

Task 2

At this stage, we have determined the IC_{50} values as an index of cytotoxicity for some of the breast tumor models already available and characterized. The data are shown in Table 2. It is apparent from comparing the isogenic neo and c-erbB-2/neu overexpressing models that increased levels of p185 led to an increase in resistance to cisplatin of about 2-fold in the MCF-7 system (neo, 0.43 μM vs. Her2-18, 0.83 μM), but there was no significant change in the mutant p53 MDA-MB-435 system (neo, 1.37 μM vs. eB1, 1.09 μM). At the same time, it is clear that the SK-Br3 model is more sensitive to cisplatin (low IC_{50}) than would be predicted from the high expression of p185. Conversely, ZR75-1 cells have wild-type p53 and have very low c-erbB-2/neu expression, but are substantially resistant to cisplatin (high IC_{50}). Thus, there is no clear relationship between p53 or c-erbB2/neu status and cisplatin cytotoxicity, and it is likely that other genetic factors may play a role in modulating the IC_{50}.

We have also evaluated a platinum analog DACH-acetato-Pt against the models, and the results are also shown in Table 2. There are two critical points to make here. First, the wild-type p53 models are more sensitive to this agent (IC_{50}, 0.12-0.85 μM) than the mutant p53 models (IC_{50}, >1 μM). It is noteworthy that the models demonstrating high levels of p185 are collaterally more sensitive (low IC_{50}) than the corresponding isogenic neo counterparts. The second point to note is that DACH-acetato-Pt is more effective than cisplatin in tumor models possessing wild-type p53 status (high cisplatin/DACH-acetato-Pt IC_{50} ratio in Table 2) than those having mutant p53 (ratio <1).

Table 2. Cytotoxicity of cisplatin and DACH-acetato-Pt against breast tumor models

Model	MCF-7 par	MCF-7/neo	MCF-7/Her2-18	ZR75-1	MDA-MB-435 par	MDA-MB-435/neo	MDA-MB-435/eB1	SK-Br3
IC_{50} (μM) Cisplatin	0.83	0.43	0.83	9.58	0.84	1.37	1.09	0.59
IC_{50} (μM) DACH-Pt	0.17	0.24	0.12	0.85	1.11	1.54	1.17	1.38
Cisplatin: DACH-Pt IC_{50} ratio	4.88	1.79	6.92	11.3	0.76	0.89	0.93	0.43

par = parental cell line. Results are shown as mean of 3-6 independent observations.

From this data, it appears reasonable to suggest that mutant p53 may confer resistance to cisplatin and DACH-acetato-Pt, but increased levels of p185 against a wild-type p53 background can confer sensitivity to breast tumor cells. Furthermore, the high level of cisplatin resistance in the wild-type p53 model ZR75-1 and its reversal by DACH-acetato-Pt is a significant finding that will be pursued further.

Task 3

The biochemical pharmacology has been determined in the wild-type p53 models, and the data are shown in Table 3. Cell uptake and DNA adduct data are derived after exposing cells to 100 μM drug, and DNA damage tolerance is defined as adducts that are required to kill 50% of the tumor cells. Overexpression of C-erbB-2/neu did not effect cellular uptake and DNA adduct levels of cisplatin or DACH-acetate-Pt in the MCF-7 model system. The values for DACH-acetato-Pt, on the other hand, were lower compared to those of cisplatin. DNA damage tolerance was increased 2-fold for cisplatin by the overexpression of c-erbB-2/neu, and it was substantially greater in ZR75-1 cells. Tolerance values for DACH-acetato-Pt were substantially lower compared to those for cisplatin. These data strongly indicate that the 2-fold increase in resistance to cisplatin by increased levels of p185 is due entirely to the increase in tolerance to cisplatin-induced adducts. Similarly, the greater sensitivity of the tumor models to DACH-acetato-Pt is due to reduced tolerance to adducts formed by this novel agent.

Table 3. Biochemical pharmacology of cisplatin and DACH-acetato-Pt in breast tumor models

	Cell Uptake (100 μM drug) (ng Pt/mg protein)		DNA adduct (100 μM drug) (ng Pt/mg DNA)		DNA Damage Tolerance (ng Pt/mg DNA)	
Model	Cisplatin	DACH-Pt	Cisplatin	DACH-Pt	Cisplatin	DACH-Pt
MCF-7/neo	145.4 ± 21.4	55.6 ± 9.1	59.9 ± 17.7	9.25 ± 3.57	15.4 ± 4.56	1.33 ± 0.51
MCF-7/Her2-18	150.0 ± 42.5	71.7 ± 17.6	66.5 ± 13.5	15.0 ± 1.49	33.1 ± 6.87	1.08 ± 0.11
ZR75-1	71.4 ± 5.27	58.0 ± 13.5	30.6 ± 7.09	20.4 ± 2.58	175.6 ± 40.8	10.4 ± 1.31

Results are shown as Mean ± SD; N = 3-5.

Tasks 6 and 7

The induction, transactivation function, and post-translational regulation of p53 was evaluated in the neo and c-erbB-2/neu overexpressing tumor cells. Induction of total p53 was seen to be dependent on dose following a 2-h drug exposure (Figure 2) and with time following a 2-h exposure to 20 μM drug concentration (Figure 3). Inductions of phosphorylated forms of p53 ($p53^{ser\text{-}15}$ and $p53^{ser\text{-}392}$) were also observed with cisplatin. DACH-acetato-Pt, in contrast, also induced $p53^{ser\text{-}15}$ but to a lesser extent. This analog, however, was a very poor inducer of $p53^{ser\text{-}392}$. The transactivation of p21 and mdm2 is evident from the Western immunoblots, but the

extent is consistent with the levels of p53 induced. However, it is clear that increased levels of p185 attenuates induction of total p53 and $p53^{ser-15}$ by cisplatin but no difference was apparent between neo and c-erbB-2/neu overexpressing cells exposed to DACH-acetato-Pt.

These results suggest that cisplatin and DACH-acetato-Pt activate independent signal transduction pathways which regulate p53, and that p185 only impinges on the pathway activated by cisplatin. These studies are ongoing to define the effects on the apoptotic genes and pathway.

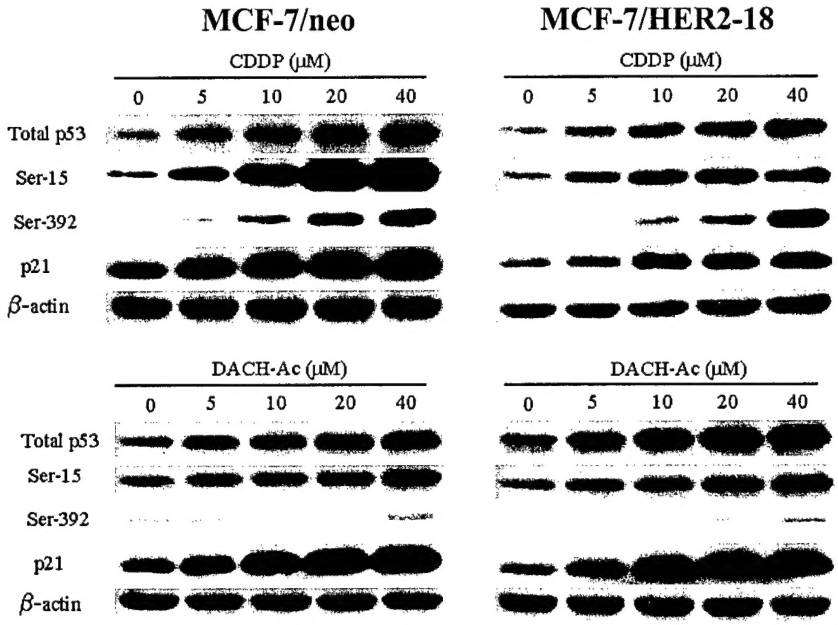

Figure 2. Concentration-dependent induction of p53 and associated proteins

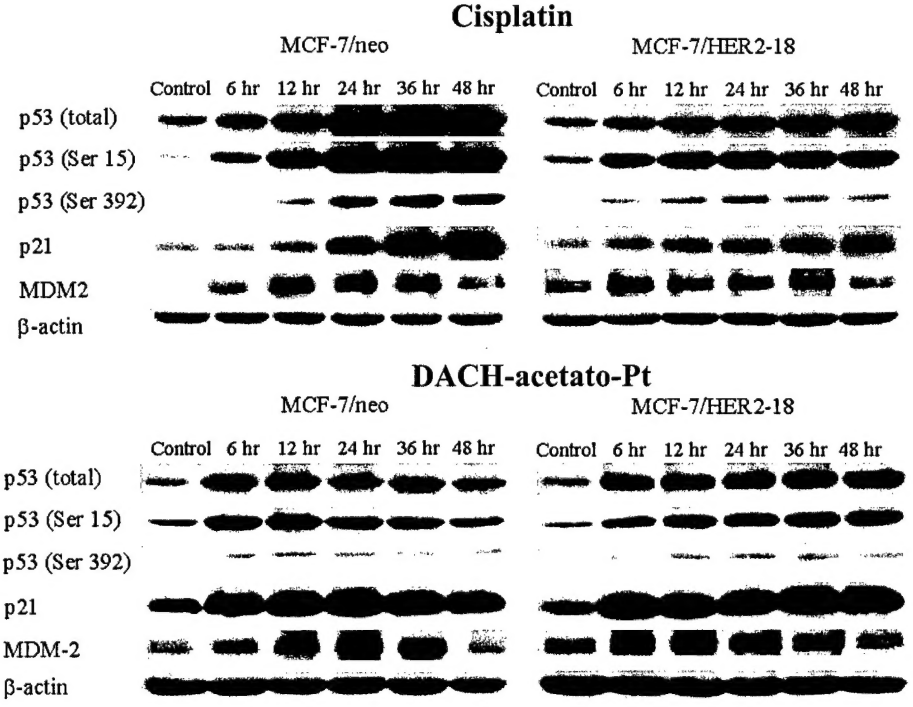

Figure 3. Time-dependent induction of p53 and associated proteins

Key Research Accomplishments

- Overexpression of c-erbB-2/neu increases resistance to cisplatin but not to the analog DACH-acetato-Pt
- Resistance to cisplatin due to an increase in DNA damage tolerance
- The differential effects of p185 on cytotoxicity of cisplatin and DACH-acetato-Pt appears to correlate with regulation of p53
- Cisplatin and DACH-acetato-Pt regulate p53 through independent signal trandsduction pathways

Reportable Outcomes

The results arising from this project have been submitted as an abstract for presentation at the next Annual Meeting of the American Association for Cancer Research in New Orleans in March 2001.

Conclusions

The results indicate that both p53 (wild-type and mutant) and overexpression of c-erbB-2/neu lead to cisplatin resistance, and that the resistance due to wild-type p53 and c-erbB-2/neu overexpression can be circumvented by DACH-acetato-Pt. The fact that wild-type p53 can lead to substantially greater resistance to an antitumor agent is a novel finding that may have greater limitations in the treatment of breast cancer. The significance of this finding, however, needs to be pursued further for a greater appreciation. The data indicate that overexpression of c-erbB-2/neu can interfere with p53 regulation when the DNA damaging agent is cisplatin, but there is no effect on regulation when the damage is induced by DACH-acetato-Pt. This suggests that this novel compound may have clinical utility in the treatment of breast cancer.

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Modulation by HER2/neu of the Cytotoxicity of Cisplatin and 1R,2R-Diaminocyclohexane-diacetato-dichloro-platinum(IV) (DACH-Acetato-Pt) Against Wild-Type p53 MCF-7 Breast Tumor Cells. M. Watanabe, J. Nakamura, K. Mujoo, A.R. Khokhar, and Z.H. Siddik. The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Wild-type p53 facilitates drug-induced apoptosis, whereas HER2/neu (HER2) induces resistance to some antitumor agents, including cisplatin, and sensitivity to others. Therefore, the aim was to study the effect of the non-cross-resistant platinum complex DACH-acetato-Pt against MCF-7/HER2-18 (HER2-18) having wild-type p53 and stably-transfected HER2 gene, and a control isogenic MCF-7/neo (neo) cell line. Basal levels of HER2 by Western analysis were 5.4-fold greater in HER2-18 compared to neo cells, and the active phosphorylated-form of HER2 was detectable in HER2-18 cells but not in neo. The HER2-18 model was 2-fold resistant to cisplatin compared to neo (IC_{50}: 0.83 vs. 0.44 μM using continuous drug exposure; 18.2 vs. 9.8 μM using 2-hour exposures). In contrast, the HER2-18 cell line demonstrated significant collateral sensitivity to DACH-acetato-Pt by up to 2-fold compared to neo (IC_{50}: 0.12 vs. 0.24 μM - continuous exposures; 15.0 vs. 22.1 μM - 2-hour exposures). DNA damage tolerance to CDDP was significantly higher in HER2-18 (12 ng Pt/mg DNA) than in neo (5.8), whereas there was no significant difference between the two models exposed to DACH-acetato-Pt (1.9-2.0 ng Pt/mg DNA). Although wild-type p53 and $p21^{Waf1/Cip1}$ (p21) were induced in neo and HER2-18 models after treatment with cisplatin, the induction was significantly less in HER2-18 cells. On the other hand, both protein molecules were similarly induced in the two cell lines after treatment with DACH-acetato-Pt. Interestingly, p53 was phosphorylated at serine-15 in a dose-dependent fashion in neo cells treated with cisplatin, but phosphorylation was suppressed in HER2-18. With DACH-acetato-Pt, this phosphorylation was very low in both cell lines. In conclusion, overexpression of HER2 induces cisplatin resistance by suppressing p53 induction, possibly through down-regulating serine-15 phosphorylation of p53. DACH-acetato-Pt, in contrast, likely activates an independent p53-mediated apoptotic pathway that is facilitated by HER2 by an unknown mechanism. The results indicate that DACH-acetato-Pt may have utility in the management of breast tumors overexpressing HER2 against a wild-type p53 background. (U.S. Army Grant DAMD17-99-1-9269).

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